

NANOENCAPSULATION OF PROBIOTIC BITTER GOURD JUICE POWDER

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ABSTRACT

Momordica charantia Linn, known as bitter gourd or karela, is a cucurbit vine native to Asia. It is a revolutionary plant for its versatility as foodstuff and therapeutic applications. It is highly nutritious in protein, ascorbic acid, calcium, iron and phosphorus contents. Charantins, a mixture of steroidal saponins that are abundant in the fruit have been proposed to contribute hypoglycemic and antihyperglycemic activity. A study was conducted to produce nanoencapsulated probiotic bitter gourd juice powder using spray dryer at three different inlet air temperatures viz., 140, 150 and 160 °C with three different encapsulating agents (i.e., 30% each of maltodextrin, gum arabic and mixture of both maltodextrin (15%) and gum arabic (15%)). The powders were analyzed for proximate compositions viz., moisture content, crude fibre, crude fat, carbohydrate and ash and functional properties viz., loose and tapped bulk density, carr's index and hausner ratio. The results showed that, increasing the inlet air temperature, moisture content, water activity and bulk density were decreased, while solubility was increased. Moisture content and water activity were significantly affected by the maltodextrin concentration whereas bulk density and solubility were negatively influenced. The highest viable counts of *L. casei* were found in powders encapsulated with maltodextrin over a storage period.

KEYWORDS: Encapsulating Agents, Bitter Gourd, Probiotics, Charantins, Antihyperglycemic Activity and Spray Drying

Received: Jan 29, 2016; **Accepted:** Feb 12, 2015; **Published:** Feb 19, 2016; **Paper Id.:** IJASRAPR20163

INTRODUCTION

Momordica charantia Linn, known as bitter melon or karela, is a cucurbit vine native to Asia (Yang and Walters, 1992). Bitter gourd is grown throughout India and is known by several names such as *Balsam pear* or *Bitter cucumber* in English, *Karela* in Hindi, Gujarathi and Punjabi, *Karala* in Marathi, *Beet Karela* in Assamese, *Kakara kaya* in Telugu, *Hagala kayi* in Kannada and *Pavakai* in Malayalam and Tamil. The total area under this crop in India during the year 2012-13 was estimated to be 78.12 thousand hectares and the production was about 883.69 thousand metric tonnes (Ministry of Agriculture, Government of India). Karnataka, Maharashtra, Tamil Nadu and Kerala are the major bitter gourd growing states in India.

It is a revolutionary plant for its versatility as foodstuff and therapeutic applications. The fruit is highly nutritious due to the presence of higher contents of protein, ascorbic acid, calcium, iron and phosphorus (Madaan and Lal, 1984; Assubaie and El-Garawany, 2004). Charantins, a mixture of steroidal saponins that are abundant in the fruit of bitter gourd, have been proposed to contribute to the hypoglycemic and antihyperglycemic activity of bitter gourd (Harinantenaina *et al.*, 2006). The extracts of fruit pulp and seed have been reported to have various

medicinal properties, including antitumor and antimutagenic activities (Jilka *et al.*, 1983). The bitter taste of bitter gourd is encapsulated using different encapsulating agent's viz., maltodextrin, gum arabic, starch etc.

MATERIALS AND METHODS

Materials

The bitter gourds (Plate 1) were procured from the local market of Raichur, Karnataka. Probiotic bacterium (*Lactobacillus casei*) and other chemicals were obtained from the MTCC (Microbial Type Culture Collection), Institute of Microbial Technology, Chandigarh, India to carry out the microbiological analysis. The additives viz., maltodextrin (DE=20), gum arabic and the other related chemicals and standards were procured from M/s Himedia, Mumbai and M/s, SD Fine, Bangalore. All the chemicals used were of analytical grade.



a) Fresh Bitter Gourd



b) Bitter Gourd Juice

Plate 1: Fresh Bitter Gourd and Juice Used for the Experimentation

Spray Drying

The spray drying process was carried out with a laboratory spray dryer (Labultima LU-222 advanced, Labultima, Mumbai) (Plate 2) using ultrasonic spray nozzle at three different inlet air temperatures viz., 140, 150 and 160 °C and at constant outlet air temperature of 85 °C with three different combination of encapsulating agents: M1 (30% maltodextrin), M2 (30% gum arabic), M3 (30% mixture of maltodextrin (15%) and gum arabic (15%)). The feed solutions containing *L. casei* were kept under magnetic agitator at room temperature and fed into the main chamber through a peristaltic pump, with a feed flow rate of 1 and 2 ml/min. The nanoencapsulated bitter gourd powder samples were collected from the cyclone. The samples were packed in polypropylene pouches and stored at room temperature.

The powders were analysed for proximate and functional properties. All the experiments were performed in triplicate. The obtained spray dried particles were also analysed to determine the viable cell counts of *L. casei* in the nanoencapsulated probiotic bitter gourd juice powder during its storage period of 4 weeks at room temperature.



Plate 2: Spray Drier Used for Production of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

Determination of Proximate and Functional Properties

The nanoencapsulated probiotic bitter gourd juice powders were analysed to determine the proximate composition *viz.*, moisture content, crude fat, crude fibre, crude protein, carbohydrates, ash by following the procedures as outlined in AOAC (2005). The functional properties *viz.*, loose bulk density, tapped bulk density; Hausner Ratio (HR) and Carr's Index (CI) of nanoencapsulated probiotic bitter gourd juice powder were determined using standard procedures as explained below.

Bulk Density

The bulk density of powder samples were measured according to the procedure described by Caparino *et al.* (2012) and Lebrun *et al.* (2012). Approximately, 1 g of powdered sample was freely poured into a 5 ml graduated glass cylinder (readable at 1 ml) without tapping and disturbance and this was measured as loose bulk density of sample. The same samples were repeatedly tapped for 20 times manually by lifting and dropping the measuring cylinder under its own weight at a vertical distance of 14 ± 2 mm height over a rubber mat until negligible difference in volume between succeeding measurements was observed and this was measured as tapped bulk density of nanoencapsulated probiotic bitter gourd juice powder (Hari *et al.*, 2013). The bulk density sample was computed by using the following formula;

$$\text{Loose bulk density (g.cc}^{-1}\text{)} = \frac{\text{Weight of powder (g)}}{\text{Bulk powdered volume (cc)}}$$

$$\text{Tapped bulk density (g.cc}^{-1}\text{)} = \frac{\text{Weight of powder (g)}}{\text{Tapped powdered volume (cc)}}$$

Flowability and Cohesiveness of Powder

The powder samples were evaluated for their flowability and cohesiveness in terms of Carr's Index (CI) and Hausner Ratio (HR), respectively. Both CI and HR were calculated from the loose bulk and tapped densities of the nanoencapsulated probiotic bitter gourd juice powder, according to formula given by Olayemi *et al.* (2008).

Carr's Index

The compressibility index of the nanoencapsulated probiotic bitter gourd juice powder was determined by Carr's Index (Compressibility Index). It is a simple test to evaluate the Carr's Index from loose bulk density and tapped density of nanoencapsulated probiotic bitter gourd juice powder. The formula for Carr's Index is as below;

$$\text{Carr's Index (\%)} = \frac{\text{Tapped bulk density (g.cc}^{-1}) - \text{Loose bulk density (g.cc}^{-1})}{\text{Tapped bulk density (g.cc}^{-1})} \times 100$$

Hausner Ratio

The Hausner Ratio is a number that is correlated to the flowability of a nanoencapsulated probiotic bitter gourd juice powder. It was calculated by using the following formula;

$$\text{Hausner Ratio} = \frac{\text{Tapped bulk density (g.cc}^{-1})}{\text{Loose bulk density (g.cc}^{-1})}$$

Survival of Nanoencapsulated *L. casei* on Spray Drying during Storage Period

The viable cell counts (*L. casei*) in the developed nanoencapsulated probiotic bitter gourd juice powder were enumerated over the storage period. The enumeration was carried out by following spread plate method with *Lactobacillus* MRS agar as a growth medium (Parveen, 2009). One gram of the sample was serially diluted in sterile distilled water until 10^{-6} dilutions were reached. About 0.1 ml aliquot from 10^{-6} dilution was transferred to the sterile petriplates containing solidified MRS agar media. Then the plates were incubated for a period of 48 h in an incubator maintained at a temperature of 32 °C. After the incubation period, the colonies were counted and the number of cfu/g of sample was calculated by applying the following formula;

$$\begin{aligned} &\text{Number of colony forming units (cfu) per g of the sample} \\ &= \frac{\text{Mean number of cfu} \times \text{Dilution factor}}{\text{Volume or weight of the sample}} \end{aligned}$$

Statistical analysis

The experimental data obtained was statistically analyzed using statistical software, Design Expert Version 7.7.0 trial version (State-Ease, Minneapolis, MN). The models generated to represent the responses were evaluated in terms of F-ratio. The effects of the independent variables on the physical properties of the powders were studied. The statistical analysis was carried out using factorial completely randomized block design (FCRD).

RESULTS AND DISCUSSIONS

The developed nanoencapsulated probiotic bitter gourd juice powder is as shown in Plate 3. The proximate

composition of nanoencapsulated probiotic bitter gourd juice powder viz., moisture content, fat, protein, carbohydrates and ash determined by standard methods was carried out thrice and the average values of proximate composition are presented in

Table 1 below. The results of proximate compositions were in accordance with Patil *et al.* (2014) on spray drying of guava powder.



Plate 3: Nanoencapsulated Probiotic Bitter Gourd Juice Powder

The nanoencapsulated probiotic bitter gourd juice powder produced with maltodextrin at lower inlet air temperature showed higher ($P < 0.05$) moisture content than that produced with the gum arabic and mixture of maltodextrin and gum arabic at the same inlet air temperatures (Table 1). These results are due to higher drying rate and decreased amount of water introduced to the drier (Masters, 1991; Perez-Munoz and Flores, 1997). The moisture content of powder ranged from 3.07 to 4.66% (d.b.) with an average of 3.99% (d.b.). The minimum moisture content of 3.07% (d.b.) was observed for treatment T_{18} i.e., sample with 30% mixture of maltodextrin (15%) and gum arabic (15%) sprayed with a feed flow rate of 1 ml/min at an inlet air temperature of 160 °C and maximum moisture content of 4.66% (d.b.) was observed in treatment T_{13} i.e., sample with 30% concentration of gum arabic sprayed with a feed flow rate of 1 ml/min and at an inlet air temperature of 140 °C. The effect of spray drying on moisture content of nanoencapsulated probiotic bitter gourd juice powder is as shown in the Figure 1.

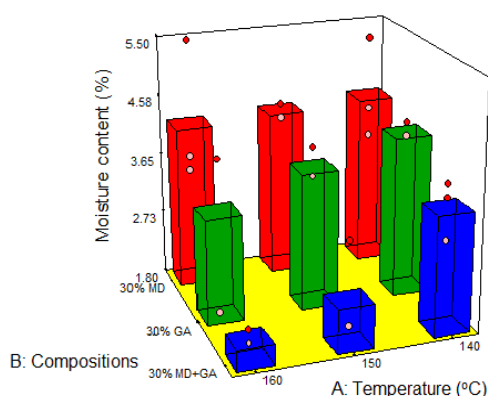


Figure 1: The Effect of Spray Drying on Moisture Content of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

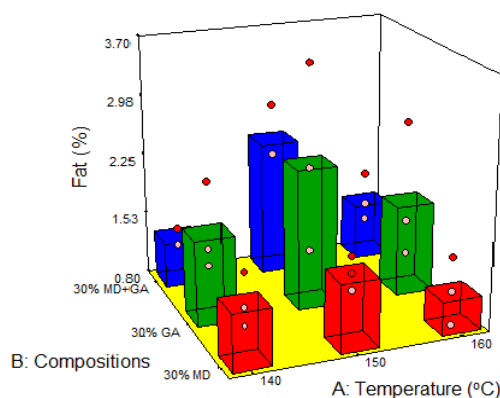


Figure 2: The Effect of Spray Drying on Fat of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

Table 1: Proximate Composition of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

Treatments	Tempe- Rature (°C)	Additives (%)	Feed Flow Rate (ml/min)	Moisture Content (%)	Crude Fat (%)	Crude Fibre(%)	Crude Protein (%)	Carbo- Hydrates (%)	Ash(%)
T ₁	140	M1	2	4.39	1.46	1.26	4.32	86.70	1.87
T ₂	150		2	4.31	1.60	1.20	3.97	87.32	1.60
T ₃	160		2	4.25	1.20	1.33	4.08	87.73	1.41
T ₄	140	M2	2	4.24	1.80	1.53	2.74	88.27	1.42
T ₅	150		2	3.88	2.46	1.46	2.51	87.96	1.73
T ₆	160		2	3.39	1.86	1.40	6.07	85.61	1.67
T ₇	140	M3	2	3.59	1.33	1.66	4.43	87.19	1.80
T ₈	150		2	3.28	2.40	1.46	4.73	86.66	1.47
T ₉	160		2	3.18	1.46	1.40	2.33	90.10	1.53
T ₁₀	140	M1	1	4.29	1.73	1.33	2.28	88.84	1.53
T ₁₁	150		1	4.23	1.66	1.26	2.21	89.24	1.40
T ₁₂	160		1	4.16	1.33	1.26	2.63	89.16	1.46
T ₁₃	140	M2	1	4.66	1.21	1.40	2.33	89.07	1.33
T ₁₄	150		1	4.57	1.26	1.53	1.83	89.08	1.73
T ₁₅	160		1	4.23	1.26	1.33	2.45	88.87	1.86
T ₁₆	140	M3	1	4.55	1.40	1.67	2.39	87.88	2.11
T ₁₇	150		1	3.62	1.41	1.26	2.28	89.90	1.53
T ₁₈	160		1	3.07	1.40	1.53	2.80	89.54	1.66
Treatments	Mean SD CV			3.99	1.57	1.41	3.13	88.28	1.61
				0.75	0.49	0.23	0.47	0.98	0.42
				19.43	30.95	16.29	15.05	33.05	25.96

M1= 30% Maltodextrin; M2= 30% Gum arabic; M3= 30% Mixture of maltodextrin and gum arabic;

The fat content of nanoencapsulated probiotic bitter gourd juice powder ranged from 1.20 to 2.46% with an average of 1.57%. The minimum fat content of 1.20% was observed in treatment T₃ *i.e.*, sample with 30% concentration of maltodextrin sprayed with a feed flow rate of 2 ml/min and at an inlet air temperature of 160 °C and maximum of 2.46% was observed in treatment T₅ *i.e.*, sample with 30% concentration gum arabic sprayed with a feed flow rate of 2 ml/min and at an inlet air temperature of 150 °C. The effect of spray drying on fat of nanoencapsulated probiotic bitter gourd juice powder is as shown in the Figure 2.

The fibre content of nanoencapsulated probiotic bitter gourd juice powder ranged from 1.20 to 1.67% with an average of 1.41%. The minimum fat content of 1.20% was observed in treatment T₂ *i.e.*, sample with 30% concentration of maltodextrin sprayed with a feed flow rate of 2 ml/min and at an inlet air temperature of 150 °C and maximum of 1.67% was observed in treatment T₁₆ *i.e.*, sample with 30% concentration of mixture of both maltodextrin and gum arabic sprayed with a feed flow rate of 1 ml/min and at an inlet air temperature of 140 °C. The effect of spray drying on fibre content of nanoencapsulated probiotic bitter gourd juice powder is as shown in the Figure 3.

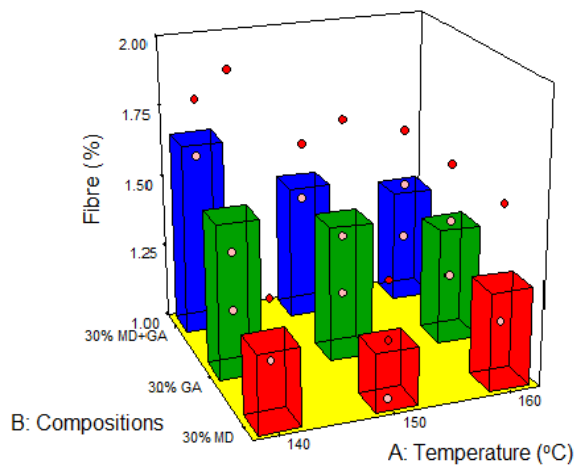


Figure 3: The Effect of Spray Drying on Fibre Content of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

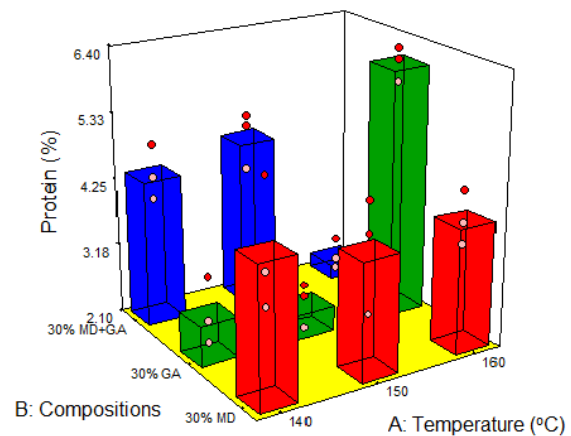


Figure 4: The Effect of Spray Drying on Protein of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

The protein content of nanoencapsulated probiotic bitter gourd powder ranged from 1.83 to 6.07% with an average 3.13%. The minimum protein content of 1.83% was observed in treatment T_{14} *i.e.*, sample with 30% concentration of gum arabic sprayed at a feed flow rate of 1 ml/min and inlet air temperature of 150 °C and maximum protein content of 6.07% was observed in treatment T_6 *i.e.*, sample with 30% concentration of gum arabic sprayed with a feed flow rate of 2 ml/min and at an inlet air temperature of 150 °C. The effect of spray drying on protein of nanoencapsulated probiotic bitter gourd juice powder is as shown in the Figure 4.

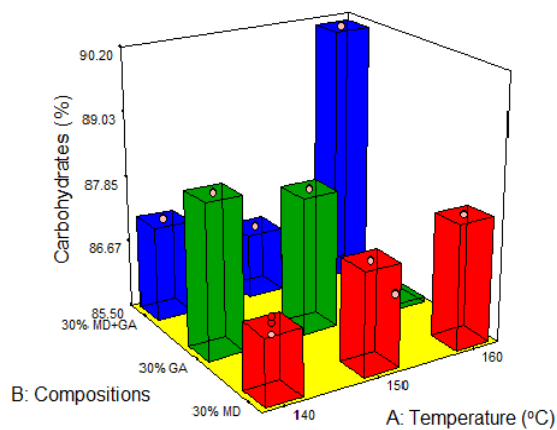


Figure 5: The Effect of Spray Drying on Carbohydrate Content Nanoencapsulated Probiotic Bitter Gourd Juice Powder

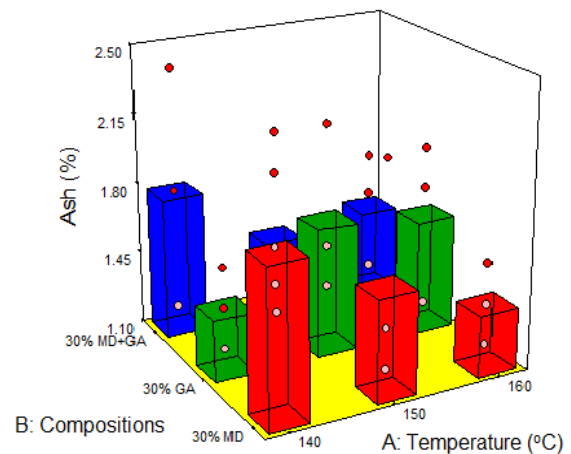


Figure 6: The Effect of Spray Drying on Ash Content Nanoencapsulated Probiotic Bitter Gourd Juice Powder

The carbohydrates content of nanoencapsulated probiotic bitter gourd juice powder ranged from 85.61% to 90.10% with an average of 88.28%. The minimum carbohydrate content of 85.61% was observed in treatment T_6 *i.e.*, sample sprayed with 30% concentration of gum arabic with a feed flow rate of 2 ml/min and at an inlet air temperature of 160 °C and maximum of 90.10% was observed in treatment T_9 *i.e.*, sample sprayed with 30% mixture of maltodextrin and gum arabic with a feed flow rate of 2 ml/min at an inlet air temperature of 160 °C. The effect of spray drying on carbohydrate content of nanoencapsulated probiotic bitter gourd juice powder is as shown in the Figure 5.

The ash content of nanoencapsulated probiotic bitter gourd powder ranged from 1.33 to 2.11% with an average of 1.61%. The minimum ash content of 1.33% was observed in treatment T₁₃ *i.e.*, sample with 30% concentration of gum arabic sprayed with a feed flow rate of 1 ml/min and at an inlet air temperature of 140 °C and maximum of 2.11% was observed in treatment T₁₆ *i.e.*, sample with 30% concentration of maltodextrin and gum arabic sprayed with a feed flow rate of 1 ml/min and at an inlet air temperature of 140 °C. The effect of spray drying on ash content nanoencapsulated probiotic bitter gourd juice powder is as shown in the Figure 6.

The functional properties *viz.*, loose bulk density, tapped bulk density; Hausner Ratio (HR) and Carr's Index (CI) of nanoencapsulated probiotic bitter gourd juice powder were presented in Table 2. The results of proximate compositions were in accordance with Patil *et al.* (2014) on spray drying of guava powder.

The loose bulk density of nanoencapsulated probiotic bitter gourd juice powder ranged from 0.32 to 0.36 g/cc with an average of 0.34 g/cc. The minimum loose bulk density of 0.32 g/cc was observed in treatment T₃ *i.e.*, sample with 30% concentration of maltodextrin sprayed with a feed flow rate of 2 ml/min and at an inlet air temperature of 160 °C and maximum of 0.36 g/cc was observed in treatment T₁₀ *i.e.*, sample with 30% concentration of maltodextrin sprayed with a feed flow rate of 1 ml/min at an inlet air temperature of 140 °C. The effect of spray drying on loose bulk density nanoencapsulated probiotic bitter gourd juice powder is as shown in the Figure 7.

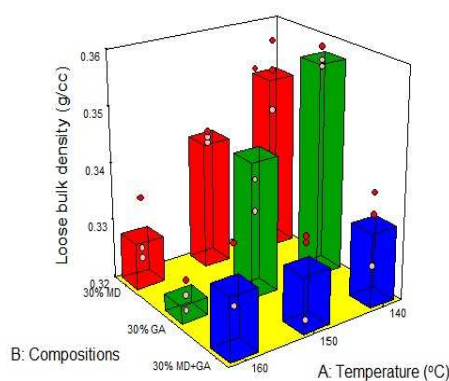


Figure 7: The Effect of Spray Drying on Loose Bulk Density of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

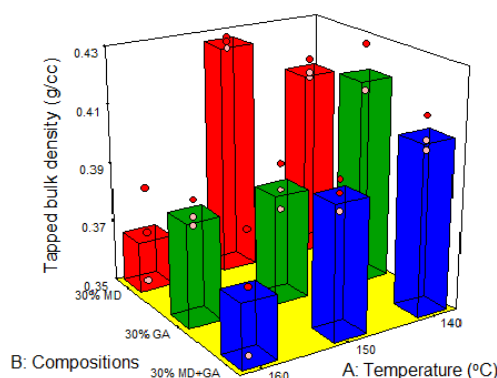


Figure 8: The Effect of Spray Drying on Tapped Bulk Density of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

The tapped bulk density of nanoencapsulated probiotic bitter gourd juice powder ranged from 0.35 to 0.43 g/cc with an average of 0.39 g/cc. The minimum of tapped bulk density of 0.35 g/cc was observed in treatment T₁₅ *i.e.*, sample with 30% concentration of gum arabic sprayed with a feed flow rate of 1 ml/min at an inlet air temperature of 160 °C and maximum of 0.43 g/cc was observed in treatment T₂ *i.e.*, sample with 30% concentration of maltodextrin sprayed with a feed flow rate of 2 ml/min and at an inlet air temperature of 150 °C. The effect of spray drying on tapped bulk density nanoencapsulated probiotic bitter gourd juice powder is as shown in the Figure 8.

Table 2: The Functional Properties of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

Treatments	Temperature (°C)	Additives (%)	Feed Flow Rate (ml/min)	LBD (g/cc)	TBD (g/cc)	Carr's Index (%)	Hausner Ratio
T ₁	140	M1	2	0.35	0.41	15.20	1.18
T ₂	150		2	0.34	0.43	20.45	1.26
T ₃	160		2	0.32	0.37	11.17	1.13

Table 2: Contd.,							
T₄	140	M2	2	0.36	0.42	14.60	1.17
T₅	150		2	0.34	0.39	11.27	1.13
T₆	160		2	0.32	0.38	17.08	1.21
T₇	140	M3	2	0.33	0.41	19.11	1.24
T₈	150		2	0.33	0.40	17.34	1.21
T₉	160		2	0.33	0.37	11.39	1.13
T₁₀	140	M1	1	0.35	0.42	14.64	1.17
T₁₁	150		1	0.35	0.41	16.66	1.20
T₁₂	160		1	0.33	0.35	4.91	1.05
T₁₃	140	M2	1	0.36	0.37	3.36	1.04
T₁₄	150		1	0.35	0.37	5.23	1.06
T₁₅	160		1	0.33	0.35	4.56	1.05
T₁₆	140	M3	1	0.33	0.36	8.32	1.09
T₁₇	150		1	0.32	0.42	18.32	1.25
T₁₈	160		1	0.31	0.36	6.59	1.07
Mean SD CV				0.34	0.39	12.23	1.15
				0.01	0.02	4.39	0.07
				1.93	5.05	35.92	5.69

M1= 30% Maltodextrin; M2= 30% Gum arabic; M3= 30% Mixture of maltodextrin and gum arabic

The Carr's Index (%) of nanoencapsulated probiotic bitter gourd juice powder ranged from 3.36 to 20.25% with an average of 12.23%. The minimum Carr's Index (%) of 3.36% was observed in treatment T₁₃ *i.e.*, sample with 30% concentration of gum arabic sprayed with a feed flow rate of 1 ml/min at an inlet air temperature of 140 °C and maximum of 20.45% was observed in treatment T₂ *i.e.*, sample with 30% concentration of maltodextrin sprayed with a feed flow rate of 2 ml/min and at an inlet air temperature of 150 °C. The effect of spray drying on Carr's Index (%) nanoencapsulated probiotic bitter gourd juice powder is as shown in the Figure 9.

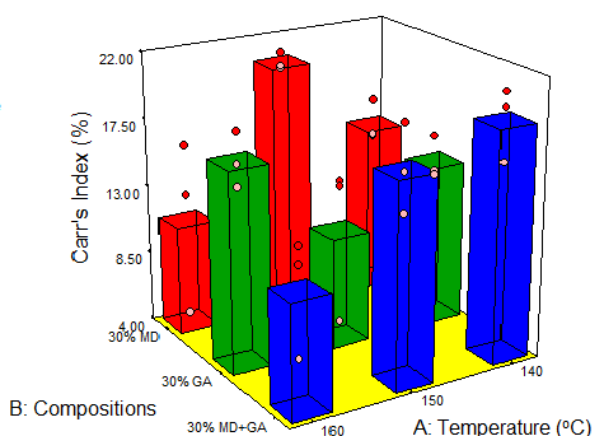


Figure 9: The Effect of Spray Drying on Carr's Index of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

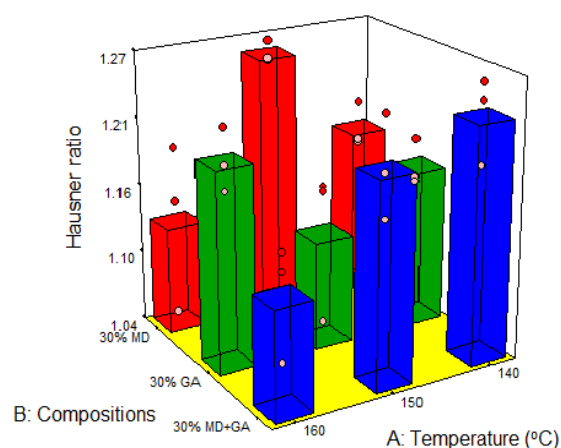


Figure 10: The Effect of Spray Drying on Hausner Ratio of Nanoencapsulated Probiotic Bitter Gourd Juice Powder

The Hausner ratio of nanoencapsulated probiotic bitter gourd juice powder ranged from 1.04 to 1.26 with an average of 1.15. The minimum Hausner ratio of 1.04 in treatment T₁₃ *i.e.*, sample with 30% concentration of gum arabic sprayed with a feed flow rate of 1 ml/min and at an inlet air temperature of 140 °C and maximum of 1.26 was observed in treatment T₂ *i.e.*, sample with 30% concentration of maltodextrin sprayed with a feed flow rate of 2 ml/min and at an inlet air temperature of 150 °C. The effect of spray drying on Carr's Index (%) nanoencapsulated probiotic bitter gourd juice

powder is as shown in the Figure 10.

Survival of Nanoencapsulated *L. casei* on Spray Drying During Storage Period

The effect of different encapsulating agents on the viability of *L. casei* in nanoencapsulated probiotic bitter gourd powder throughout storage is shown in Figure 11. The spray dried particles containing *L. casei* showed high survival up to its first week of storage encapsulated with maltodextrin than that when compared to gum arabic and mixture of both maltodextrin and gum arabic. In addition, the counts of viable probiotic cells were above the recommended levels for probiotic food throughout the whole storage time, i.e., equal to or greater than 6 log cfu/g of the product. The capsules produced with maltodextrin and gum arabic showed higher ($P < 0.05$) count, when compared to the capsules produced with mixture of both maltodextrin and gum arabic. This suggests that maltodextrin had a positive effect on the protection of *L. casei* during the encapsulation process, probably because it acts as a thermoprotector for the cells undergoing the drying process.

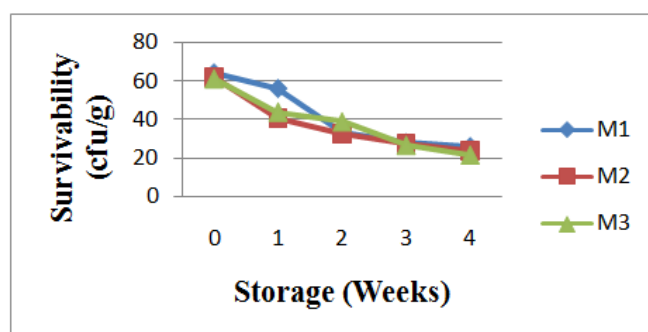


Figure 11: Survivability of *L. casei* Encapsulated With Different Encapsulating Agents

CONCLUSIONS

The spray drying of bitter gourd juice using different encapsulating agents did not affect proximate and functional properties of the powdered particles. They also help in maintaining stability of the powders during storage by decreasing the moisture content. The powders encapsulated with maltodextrin exhibited a lower bulk density than that obtained with mixture of maltodextrin and gum arabic. Finally, the results showed that the viability of the *L. casei* in the developed nanoencapsulated probiotic bitter gourd juice powder is considerably good when encapsulated with maltodextrin in turn retaining the powder properties.

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